blood®
Letter to Blood

TO THE EDITOR:

Increased prevalence of *CRLF2* rearrangements in obesity-associated acute lymphoblastic leukemia

Steven D. Mittelman,[1] Jiyoon Kim,[2] Gordana Raca,[3,4] Gang Li,[2] Matthew J. Oberley,[5] and Etan Orgel[4,6]

[1]Division of Pediatric Endocrinology, University of California, Los Angeles (UCLA) Children's Discovery and Innovation Institute, David Geffen School of Medicine, and [2]Department of Biostatistics and Computational Medicine, Jonathan and Karin Fielding School of Public Health, UCLA, Los Angeles, CA; [3]Center for Personalized Medicine, Children's Hospital Los Angeles, Los Angeles, CA; [4]Department of Pediatrics, Keck School of Medicine of the University of Southern California, Los Angeles, CA; and [5]Department of Pathology and Laboratory Medicine and [6]Cancer and Blood Disease Institute, Children's Hospital Los Angeles, Los Angeles, CA

Rearrangements in cytokine receptor–like factor 2 (*CRLF2r*) represent a subset of B-cell acute lymphoblastic leukemia (B-ALL) that demonstrates poorer survival in children and adults.[1,2] An increased incidence of acute lymphoblastic leukemia (ALL) with *CRLF2r* has been consistently reported in patients of Latinx ethnicity,[1-3] likely contributing to poorer outcomes.[3] Separately, Hispanic/Latinx populations exhibit high rates of obesity,[4] a host factor independently associated with the risk for developing B-ALL[5,6] and poorer survival.[7,8] Obesity may promote leukemogenesis and impair disease response via multiple mechanisms, including hormones, adipokines, and direct interactions with adipocytes[9-14]; however, no studies to our knowledge have examined whether obese patients are at a higher risk of developing any specific genomic subtype of ALL. Indeed, despite the higher rates of obesity in the Hispanic/Latinx population, and the connection of obesity with B-ALL, prior studies of *CRLF2r* ALL and ethnicity have not explored the potentially confounding influence of high rates of obesity in the Hispanic/Latinx population. We hypothesized that obesity contributes to preferential development of *CRLF2r* in B-ALL, and that high rates of obesity in Hispanic/Latinx populations may explain some of the observed association between ethnicity and *CRFL2r* ALL.

To test this, we collected data from 3 consecutive prospective trials (2011-2020) examining body composition in patients with newly diagnosed ALL (supplemental Table 1, available on the *Blood* Web site).[15,16] Fat mass (FM) and body fat (BF) percentage (BF%) were measured using whole-body dual-energy X-ray absorptiometry.[15] Body mass index (BMI) percentile (BMI%) was classified using population norms (obese, BMI ≥95th percentile, BMI ≥30.0 for age ≥20 years). Based on preclinical data showing interactions between adipocytes and ALL, FM (adipocyte burden) was analyzed as the primary measure of obesity. Both BMI% and BF% were also analyzed, as BMI% is generally accessible, albeit imprecise, in ALL populations,[15] and BF% is most closely associated with obese physiology.

To determine the presence of *CRLF2r*, all patients diagnosed since 2016 were evaluated with the following combination of techniques. Flow cytometry was used to detect *CRLF2* expression. Fluorescent in situ hybridization (FISH) was performed using *CRLF2* (Cytocell, Cambridge, United Kingdom) and *IGH* (Abbott Molecular Inc, Des Plaines, IL) breakapart probes; cases positive for *IGH* and *CRLF2* rearrangements were classified as *IGH-CRLF2*. If FISH was positive for a loss of 5′ *CRLF2* signal and negative for an *IGH* rearrangement, the case was classified as *P2RY8-CRLF2*. For these cases, chromosomal microarray confirmed a deletion in the Xp22.3/Yp11.3 pseudoautosomal region between the *P2RY8* and *CRLF2* genes. A proprietary platform[17] for amplification-based next-generation sequencing of tumor DNA and RNA identified concurrent IKZF1, JAK, and interleukin 7 receptor mutations, which further supported the presence of a *CRLF2r* and directly detected the *P2RY8-CRLF2* fusion. Prior to 2016, *CRLF2r* was identified on banked aspirate specimens using the same FISH approach. All pathologic diagnoses of *CRLF2r* were centrally reviewed. Following distributional analyses, locally weighted scatterplot smoothing (bandwidth, 0.8) was used to depict the smoothed average of the proportion of *CRLF2r* ALL cases by presenting FM. Linear and logistic regression models, adjusting for ethnicity and age, were constructed to, respectively, analyze the association of demographics with FM at diagnosis and FM with presence of *CRLF2r*. All analyses were 2-sided ($P < .05$) and calculated using R (www.r-project.org). All studies were approved by the institutional review board and informed consent was obtained from all participants.

Ninety-seven B-ALL patients ≥10 years old were included (supplemental Table 1). Of these, 27 of 97 (28%) were unevaluable due to technical difficulties performing FISH on the banked specimens. There were no significant differences in body composition or ethnicity for unevaluable cases nor between testing cohorts (supplemental Tables 2 and 3). Of those evaluable, 23 of 70 (33%) were diagnosed as *CRLF2r* ALL (16 of 23 *IGH-CRLF2*; 6 of 23 *P2RY8-CRLF2*; 1 of 23 *CRLF2* to unknown). Ten (44%) of the 23 CRLF2 cases had concurrent IKZF1 deletions, 3 (13%) had JAK mutations, and 3 (13%) had interleukin 7 receptor mutations.

Patients with *CRLF2r* ALL presented with significantly higher FM, BF%, and BMI% (Table 1). Even within the subset of Hispanic/Latinx subjects, FM and obesity were associated with prevalence of *CRLF2r* (Figure 1). Obesity was similarly more prevalent in those with vs without *CRLF2r* (supplemental Figure 1). As expected, Hispanic/Latinx ethnicity was associated with a higher prevalence of obesity (supplemental Table 4) and was positively correlated with FM (β = 14.51; 95% confidence interval [95%

Table 1. Description of cohort

Covariable	Total cohort		*CRLF2r* ALL		Not *CRLF2r* ALL		*P**
	n	%	N	%	n	%	
Total	70	100	23	33	47	67	
Sex							
Female	28	40	5	22	23	49	.039
Male	42	60	18	78	24	51	
Age at diagnosis, y							
Mean (SD)	15.2 (2.9)		16.2 (2.0)		14.8 (3.2)		.057
<15	30	43	5	22	25	53	.020
≥15, AYA	40	57	18	78	22	47	
Ethnicity							
Not Hispanic/Latinx	16	23	3	13	13	28	.232
Hispanic/Latinx	54	77	20	87	34	72	
Body composition, by DXA, mean (SD)							
FM, kg	23.8 (13.6)		34.1 (13.2)		18.9 (10.8)		<.0001
BF, %	31.8 (9.5)		37.3 (8.0)		29.1 (9.2)		.0007
BMI, percentile†							
Mean (SD)	76.9 (29.7)		91.3 (17.8)		69.9 (31.9)		.004
Not obese, <95th	39	56	7	30	32	68	.003
Obese, ≥95th	31	44	16	70	15	32	

AYA, adolescent and young adult; DXA, whole body dual-energy X-ray absorptiometry; SD, standard deviation.
*Fisher exact test or Student *t* test significance: $P < .05$.
†Obesity defined as sex/age adjusted BMI percentile ≥95% according to Centers for Disease Control and Prevention pediatric norms.

CI], 7.12-21.90; $P = .0002$). In multivariable analysis adjusting for ethnicity, the odds of being *CRLF2r* increased by ~13% for every kg of FM (odds ratio, 1.125; 95% CI, 1.056-1.198; $P = .0003$). As prevalence of *CRLF2r* is increased in adolescents and young adults,[2,18] adjusting for age in addition to ethnicity yielded similar effects of obesity on CRLF2r risk (odds ratio, 1.119; 95% CI, 1.049-1.194; $P = .0007$).

We report here the new finding of an association between body fat and *CRLF2r* ALL in older children and adolescents. To our knowledge, this is the first description connecting obesity to a specific high-risk genomic variant in ALL. Increased prevalence of *CRLF2r* likely contributes to the poorer survival rates observed in obese patients with B-ALL.[7,19] As obesity has recently been reported to increase the risk for developing B-ALL in Hispanic/Latinx populations,[5] it is likely that obesity may also contribute to the association between Hispanic/Latinx ethnicity and *CRLF2r* ALL.[1-3] How obesity predisposes to *CRLF2r* in this group remains unknown. A recent genome-wide association study found that *GATA3* variants were associated with risk for developing *CRLF2r* ALL and that the penetrance of the risk-associated allele was higher in Hispanic/Latinx populations.[20,21] Indeed, this same *GATA3* polymorphism contributes to altered adipogenesis and diabetes risk,[22,23] implicating a common susceptibility pathway. Alternatively, one might hypothesize that Hispanic/Latinx patients may be genetically susceptible to developing the *CRLF2r* variant of B-ALL, and obesity-induced signaling of phosphatidylinositol 3-kinase(PI3K)/AKT and mammalian target of rapamycin (mTOR) leukemia[24] provides a "second hit" that promotes survival and propagation of the *CRLF2r* leukemic clone. Improved

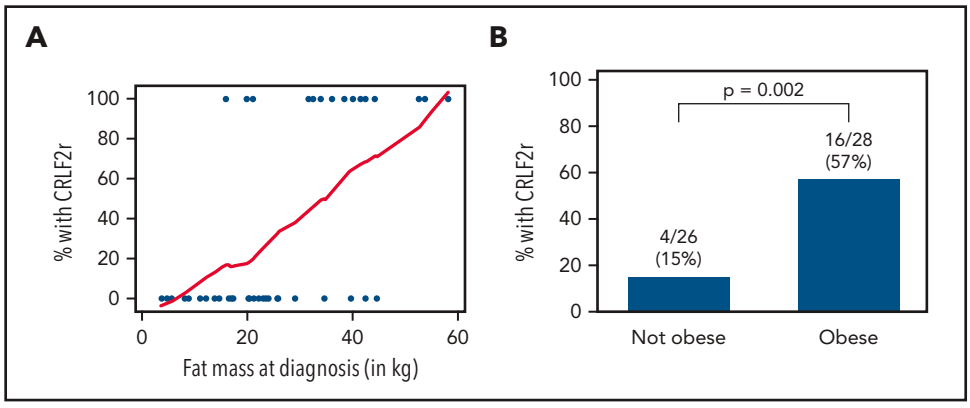

Figure 1. Association of BF and obesity with *CRLF2r* ALL in Hispanic/Latinx patients. (A) In Hispanic/Latinx patients newly diagnosed with B-ALL, the proportion of those with *CRLF2r* vs non-*CRLF2r* increased with FM (kg) as measured by whole-body dual-energy X-ray absorptiometry. Blue dots represent each case (with/without *CRLF2r*); the red line represents the moving smoothed average for proportion of cases with *CRLF2r* by increasing FM (x-axis). (B) *CRLF2r* was similarly more prevalent in Hispanic/Latinx patients who were obese (BMI ≥95%) vs those who were nonobese (BMI <95%).

understanding of the interaction of genetic predisposition, ethnicity, and obesity with development of *CRLF2r* ALL may provide critical clues to preventing or treating this high-risk subtype of ALL.

This study has several strengths. Foremost, all patients underwent prospective and detailed measurement of BF, negating reliance on BMI as a surrogate and imprecise measure and allowing for quantification of the risk derived from increasing amounts of FM. Second, most patients received comprehensive testing for *CRLF2r* and concurrent mutations. Third, although our study was not powered to detect a direct association with Hispanic/Latinx, the rates of *CRLF2r* ALL found in aggregate and in the Hispanic/Latinx population are similar to that previously reported,[1-3,18] further supporting the generalizability of these findings. These strengths were balanced by several innate limitations. Prior to 2016, *CRLF2r* was identified by FISH only; although FISH was 100% concordant with other testing, we cannot exclude the possibility, however unlikely, that flow cytometry or genomic analysis may have uncovered evidence of a cryptic *CRFL2r*. Additionally, as the trials only enrolled older children with ALL, we could not explore the impact from obesity at a younger age (ie, <10 years old). Finally, we acknowledge that to include precise measures of body composition, these findings were derived from a series of prospective trials with a relatively small sample size and validation is now required within larger cohorts.[3,18] Nonetheless, these findings offer new insights into the complex relationship between ethnicity and *CRLF2r*. As a potentially modifiable risk factor, obesity offers a unique opportunity for prevention and/or early intervention in those with inherited susceptibility to a high-risk form of B-ALL.

Acknowledgments

The authors thank Gerald Lu and Michael Lu from the Center of Personalized Medicine for their work on the *CRFL2r* testing. The authors thank the participants in the studies and their families, as well as the sponsors of this research (the Leukemia & Lymphoma Society, Gabrielle's Angel Foundation for Cancer Research, and the National Institutes of Health, National Cancer Institute).

This work was supported by the Gabrielle's Angel Foundation for Cancer Research and by Leukemia & Lymphoma Society grant LLS624911. This study was also supported, in part, by National Institutes of Health grants R01 CA201444 (S.D.M.), P30 CA16042 and P50 CA2110 (G.L.) from the National Cancer Institute, and UL1TR000124 (G.L.) from the National Center for Advancing Translational Sciences. This work was also supported, in part, by National Institutes of Health, National Center for Advancing Translational Sciences grants UL1TR001855 and UL1TR000130 via the Southern California Clinical and Translational Science Institute.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Authorship

Contribution: E.O. was responsible for study conception; E.O. and S.D.M. designed the study; G.R. and M.J.O. were responsible for central review of hematopathology; E.O., S.D.M., J.K., and G.L. verified data; J.K. and G.L. analyzed data; and all authors interpreted data, revised the manuscript, and gave final approval of the manuscript.

Conflict-of-interest disclosure: E.O. was on a scientific advisory board for Jazz Pharmaceuticals outside of the scope of this work. The remaining authors declare no competing financial interests.

ORCID profiles: S.D.M., 0000-0003-2867-1298; G.R., 0000-0002-3521-9585; M.J.O., 0000-0001-6419-2513; E.O., 0000-0002-1487-6818.

Correspondence: Etan Orgel, Cancer and Blood Disease Institute, Children's Hospital Los Angeles, 4650 Sunset Blvd, MS#54, Los Angeles, CA 90027; e-mail: eorgel@chla.usc.edu.

Footnotes

Submitted 11 February 2021; accepted 2 April 2021; prepublished online on Blood First Edition 19 April 2021.

Deidentified data are available upon reasonable request for 3 years from eorgel@chla.usc.edu.

The online version of this article contains a data supplement.

REFERENCES

1. Reshmi SC, Harvey RC, Roberts KG, et al. Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's Oncology Group. *Blood.* 2017;129(25):3352-3361.
2. Jain N, Roberts KG, Jabbour E, et al. Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. *Blood.* 2017;129(5):572-581.
3. Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood.* 2010;115(26):5312-5321.
4. Claire Wang Y, Gortmaker SL, Taveras EM. Trends and racial/ethnic disparities in severe obesity among US children and adolescents, 1976-2006. *Int J Pediatr Obes.* 2011;6(1):12-20.
5. Ghosh T, Richardson M, Gordon PM, Ryder JR, Spector LG, Turcotte LM. Body mass index associated with childhood and adolescent high-risk B-cell acute lymphoblastic leukemia risk: a Children's Oncology Group report. *Cancer Med.* 2020;9(18):6825-6835.
6. Castillo JJ, Reagan JL, Ingham RR, et al. Obesity but not overweight increases the incidence and mortality of leukemia in adults: a meta-analysis of prospective cohort studies. *Leuk Res.* 2012;36(7):868-875.
7. Butturini AM, Dorey FJ, Lange BJ, et al. Obesity and outcome in pediatric acute lymphoblastic leukemia. *J Clin Oncol.* 2007;25(15):2063-2069.
8. Orgel E, Tucci J, Alhushki W, et al. Obesity is associated with residual leukemia following induction therapy for childhood B-precursor acute lymphoblastic leukemia. *Blood.* 2014;124(26):3932-3938.
9. Orgel E, Sea JL, Mittelman SD. Mechanisms by which obesity impacts survival from acute lymphoblastic leukemia. *J Natl Cancer Inst Monogr.* 2019;2019(54):152-156.
10. Yun JP, Behan JW, Heisterkamp N, et al. Diet-induced obesity accelerates acute lymphoblastic leukemia progression in two murine models. *Cancer Prev Res (Phila).* 2010;3(10):1259-1264.
11. Hino M, Nakao T, Yamane T, Ohta K, Takubo T, Tatsumi N. Leptin receptor and leukemia. *Leuk Lymphoma.* 2000;36(5-6):457-461.
12. Behan JW, Yun JP, Proektor MP, et al. Adipocytes impair leukemia treatment in mice. *Cancer Res.* 2009;69(19):7867-7874.
13. Pramanik R, Sheng X, Ichihara B, Heisterkamp N, Mittelman SD. Adipose tissue attracts and protects acute lymphoblastic leukemia cells from chemotherapy. *Leuk Res.* 2013;37(5):503-509.
14. De Pergola G, Silvestris F. Obesity as a major risk factor for cancer. *J Obes.* 2013;2013:291546.
15. Orgel E, Mueske NM, Sposto R, Gilsanz V, Freyer DR, Mittelman SD. Limitations of body mass index to assess body composition due to

sarcopenic obesity during leukemia therapy. *Leuk Lymphoma.* 2018;59(1):138-145.

16. Orgel E, Framson C, Buxton R, et al. Caloric and nutrient restriction to augment chemotherapy efficacy for acute lymphoblastic leukemia: the IDEAL trial. *Blood Adv.* 2021;5(7):1853-1861.

17. Hiemenz MC, Ostrow DG, Busse TM, et al. OncoKids: a comprehensive next-generation sequencing panel for pediatric malignancies. *J Mol Diagn.* 2018;20(6):765-776.

18. Stock W, Luger SM, Advani AS, et al. A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403. *Blood.* 2019;133(14):1548-1559.

19. Orgel E, Genkinger JM, Aggarwal D, Sung L, Nieder M, Ladas EJ. Association of body mass index and survival in pediatric leukemia: a meta-analysis. *Am J Clin Nutr.* 2016;103(3):808-817.

20. Perez-Andreu V, Roberts KG, Xu H, et al. A genome-wide association study of susceptibility to acute lymphoblastic leukemia in adolescents and young adults. *Blood.* 2015;125(4):680-686.

21. Koller PB, Zhang H, Kantarjian H, et al. GATA3 rs3824662A allele in B-cell acute lymphoblastic leukemia in adults, adolescents and young adults: association with CRLF2 rearrangement and poor prognosis. *Am J Hematol.* 2021;96(3):E71-E74.

22. Huda N, Hosen MI, Yasmin T, Sarkar PK, Hasan AKMM, Nabi AHMN. Genetic variation of the transcription factor GATA3, not STAT4, is associated with the risk of type 2 diabetes in the Bangladeshi population. *PLoS One.* 2018;13(7):e0198507.

23. Almuraikhy S, Kafienah W, Bashah M, et al. Interleukin-6 induces impairment in human subcutaneous adipogenesis in obesity-associated insulin resistance. *Diabetologia.* 2016;59(11):2406-2416.

24. Chen J. Multiple signal pathways in obesity-associated cancer. *Obes Rev.* 2011;12(12):1063-1070.

DOI 10.1182/blood.2021011106